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THE PROSPECTS OF PROLONGED

ORGAN XENOGRAFT FUNCTION

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Clinical xenotransplantation is the diamond inside the protective humoral immunity shell that has been so hard to crack. How difficult is it going to be to shape the diamond if we break through to it? We do not know for discordant species combinations because it has been impossible to breach the humoral barrier and find out. However, we do have information about concordant combinations such as hamster to rat and chimpanzee and baboon to human.

GRAFT ACCEPTANCE: A NEW PERSPECTIVE

What happens when organ allografts or xenografts survive chronically is shown in Figure 1. Passenger leukocytes which originally come to the organs from the bone marrow promptly migrate throughout the recipient and survive after transplantation (donor cell chimerism) and are replaced in the graft with a reverse traffic of recipient leukocytes. With success, the coexisting immunocyte populations induce non-reactivity, each to the other (1).

The recent discoveries leading to this realization have been a scientific epiphany. The dominant event in the resulting 2-way immune reaction of organ transplantation is of course the host versus graft reaction (rejection) which gathers force at first, but then wanes in successful cases. The graft versus host reaction mounted by the smaller passenger leukocyte population is

not clinically detectable unless it causes graft vs host disease but it goes through the same waxing and waning transition. This component can be safely augmented by infusing bone marrow cells from the organ donor as shown in Figure 1 for the heart providing the recipient is not cytoablated. The reason is that the coexisting immune reactions are partially cancelling by gradually inducing non-reactivity, each to the other, in various stages of peripheral tolerance.

The reciprocal influence of the 2 cell populations also explains why HLA (tissue) matching is so poorly predictive of outcome (2). With a severe tissue mismatch, the genetically determined strong rejection is mitigated by the GVH. Thus, there is an escalating bidirectional immune reaction with successively greater mismatches. Yet, the clinical success rate with allografts is surprisingly similar throughout the whole spectrum of histocompatibility, being reliably increased in actual experience only when there is a perfect match. There is considerable evidence that the same events must occur for human acceptance of an animal organ.

INFECTIOUS DISEASE IMPLICATIONS OF XENOTRANSPLANTATION

The spectacular infectivity of infested organs is easy to understand in the context of the foregoing migratory phenomena. The missionary donor white cells travel to every part of the recipient body, to which they may carry viruses. This is not a reason for hand wringing. Donor screening and postoperative infectious disease monitoring have been the empirical solutions to such problems throughout the whole history of conventional allotransplantation. The same will be true for xenotransplantation.

THE ROLE OF IMMUNOSUPPRESSION

For the desired outcome from these donor-recipient cellular interactions, an umbrella of immunosuppression is necessary (Figure 1), probably for life in some recipients. However, there are numerous examples of successful drug discontinuance in patients and animals (1), even in recipients of xenografts. Two years ago in London, Ontario, David Grant and Cal Stiller transplanted the liver of a baboon to a rhesus monkey with a modification of the treatment strategy developed by Murase et al (3) in the laboratory and used for our 2 recent baboon to human liver transplantations (see below). One year ago, all immunosuppression was stopped. The still healthy chimeric rhesus monkey is proof of the feasibility of xenotransplantation in subhuman primates. There are other examples of long xenograft survival in the baboon --> monkey combination (Loma Linda, Milan,

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and Oklahoma City), but the lasting xenograft tolerance in the Canadian experiment has been a unique achievement thus far.

Thus, the drugs that we use in the transplantation clinics or laboratories are only handmaidens that permit or encourage the expression of a normal response capability of both the interacting donor and recipient immune systems. The orchestra leader in the symphony of graft acceptance is the dendritic cell (4), the antigen presenting leukocyte discovered by Steinman and Cohn at Rockefeller University in 1973 (5).

The treatment used for the 2 Pittsburgh liver xenotransplantations (6,7) consisted of a four-drug cocktail: FK 506 (now tacrolimus) given intravenously or orally, cyclophosphamide intravenously and orally, prednisone, and prostaglandin E₁. The striking synergism of cyclophosphamide and FK 506 had been demonstrated in experiments in hamster to rat heart and liver xenotransplantation (3). Untreated rat recipients of hamster livers survive for 7 days before being destroyed by combined cellular and humoral rejection. This survival is increased to 35 days with the T-cell directed FK 506 given alone. In contrast, survival is prolonged only slightly using monotherapy with cyclophosphamide or other antimetabolites such as brequinar or mycophenolate mofetil which suppress B-cell antibody responses. However, FK 506 combined with a short course

of any of the three anti-metabolite drugs gave uniform 100 day survival after hamster to rat liver or heart xenotransplantation. The animals were proven to be chimeric. Thus, the combination of the T-cell directed FK 506 and the B-cell suppressing antimetabolites was the linchpin of the clinical protocol to which the prednisone and perioperative prostaglandin were added.

THE PITTSBURGH LIVER XENOTRANSPLANTATIONS

The first patient had the best course (6). He became jaundice free for most of the 70 days of his survival. However, his canalicular enzymes were increased from the second week onward to a peak alkaline phosphatase of over 10,000 IU, suggesting biliary obstruction. Serum transaminases, an indicator of liver cell injury, were not greatly elevated at any time. At autopsy at 70 days, the entire biliary tree was plugged by inspissated bile and most of the intrahepatic ducts were denuded of epithelium. The findings were essentially the same in the second case. In addition, both recipients died of infection and both developed renal failure. Most importantly, neither achieved normal liver function.

The failure in the 2 cases was not explained by either vascular or cellular rejection. Only one of the 7 biopsies obtained from Patient 1 (this on day 12) had evidence of cellular

rejection by conventional criteria, and this was mild and focal. On day 64 after several hypotensive episodes, there was some centrilobular hepatocyte drop out. No definite evidence of cellular rejection was seen in any of the 7 biopsy samples taken from Patient 2 over a 26 day period. These benign findings were in striking contrast to the fierce cellular rejection found in 6 baboon kidney grafts 6 to 60 days after renal xenotransplantation under azathioprine and prednisone in 1963 and early 1964 (8).

The recent liver xenografts also were entirely free of the arteritis that had been seen in all previous baboon to human kidney or heart grafts. There was no trace of the occlusive endotheliolitis that appeared to be responsible for patchy gangrene of the 1963 kidney xenografts and in the baboon heart of Bailey's Baby Fae case (9). In these cases, the necrotic tissue was interspersed between islands of still functional parenchyma. In contrast, the transplanted baboon livers appeared grossly normal at surgical re-exploration shortly before death at 70 and 26 days.

The livers, which were too small for their human recipients, regenerated up to an appropriate larger size within 2-3 weeks. Finally, widespread chimerism was demonstrated with polymerase chain reaction (PCR) studies of tissues retrieved at autopsy 70 and 26 days after operation.

With so many favorable findings, why were these efforts unsuccessful? Extensive analyses and interpretations have been published elsewhere (6,7). In both cases, the conventional lymphocytotoxic crossmatch of the recipient sera with their donor lymphocytes was positive initially but negative after dithiothreitol treatment, meaning that the preformed antibodies were largely IgM. The conventional crossmatches became negative postoperatively. One hour after revascularization, sludging as well as the presence of a few polymorphonuclear leukocytes was seen in the sinusoids of the xenografts.

Both the sludging and the appearance of polys were compatible with the diagnosis of an aborted hyperacute rejection. Complement studies were consistent with this possibility. Total complement was depleted for almost 2 weeks while complement components C₃, 4, and 5 became undetectable. During this time, circulating immune complexes appeared. These complement changes were similar to those in recipients of allografts which escaped hyperacute rejection despite transplantation across a positive lymphocytotoxic crossmatch (discussed in 7).

Although the biopsies were thought to be normal at first examination, closer inspection showed a very fine microsteatosis in the hepatocytes of both xenografts which became progressively more obvious in the second case over the next few days.

Microsteatosis also has been reported in cases of human to human liver transplantation carried out in the presence of positive cytotoxic crossmatch and in cases in which hepatic allografts have had inexplicable primary non-function. IgM and IgG binding was found in the baboon xenografts. The IgM largely disappeared from the graft tissues by 24 days but significant amounts of IgG remained.

CONCLUSION FROM BABOON CLINICAL TRIALS

The evidence was that our 2 patients had encountered a slow motion version of the hyperacute rejection described earlier this morning by Dr. Platt. This sobering conclusion prompted us to cancel the last 2 cases of our IRB-approved series of 4 (7). We have no clinical protocols current or pending. Instead, we are exploring strategies in the laboratory to deal with the problem of subtle complement activation, whether antibody initiated (classical pathway) or not (alternative pathway). As discussed elsewhere (7), this special kind of rejection was first recognized in both allograft and xenograft recipients more than 30 years ago. The problem was thus a familiar one for which a satisfactory solution had not been found (i.e. in highly sensitized kidney allograft candidates) despite persistent efforts by many people. Now we knew that the practical application of xenotransplantation, even with the use of

concordant donor species was not going to be possible without resolving the historically intractable problem of humoral rejection. Fine tuning of available agents might permit occasional success in humans with the baboon donor, but the field (including the use of distant species such as the pig) would be frozen without some therapeutic ingredient which either was not available or had not been tried.

REGULATORY ISSUES

A policy conference was convened on June 25-27, 1995, to determine, first, if and secondarily, how, to foster, apply, and regulate xenotransplantation technology. It seems to me that the only immediate question is whether to foster these efforts. The doomsday arguments voiced by some of the participants about creating new infectious disease syndromes with clinical trials might have been credible a third of a century ago. Such concerns largely have been defused by the return of tens of thousands of immunosuppressed organ recipients to their pets, farms, homes, health care professions, ministries, public office, and other ways of life. The patients did not prove to be walking time bombs who introduced or spread HIV, the Ebola epidemic, or the Marburg virus. I know of no single such example in the now huge transplant recipient population. A decision whether to foster xenotransplant developments should not hinge on the possibility

of causing a modern day plague. From what we have learned at a practical level, that risk, while undeniable, is miniscule.

We are discussing here an ethical subject which concerns the relationship of humans to animals at spiritual and other levels. Therefore, the probity of xenotransplantation or the lack of it is an issue for all of society to decide, not any small outspoken fraction of it or even the medical profession. I might add here that I respect and take seriously those who are opposed to research and development in xenotransplantation. Scientists and physicians or surgeons like me tend to be lined up on the other side. We also are a small and noisy group. We cannot forget that we are the servants, not the masters, of those who need our medical services, or someday might. Thus, we need a signal from the public at large about desisting in these efforts, or proceeding.

As for application and regulation, there is nothing in my opinion to apply at a practical level or to regulate now, or in the near future. The science simply is not that far advanced. Regulating something that does not exist is the best way I know to ensure that it never will exist. Governance of the very limited clinical research that is justified at the present time should not be burdensome for the legislatively mandated IRB's at a local level. This established framework should be

buttressed by the formation of national resource groups which can provide expertise and guidelines. No case should ever be done without open disclosure, a condition that will necessitate registration of all xenotransplant cases.

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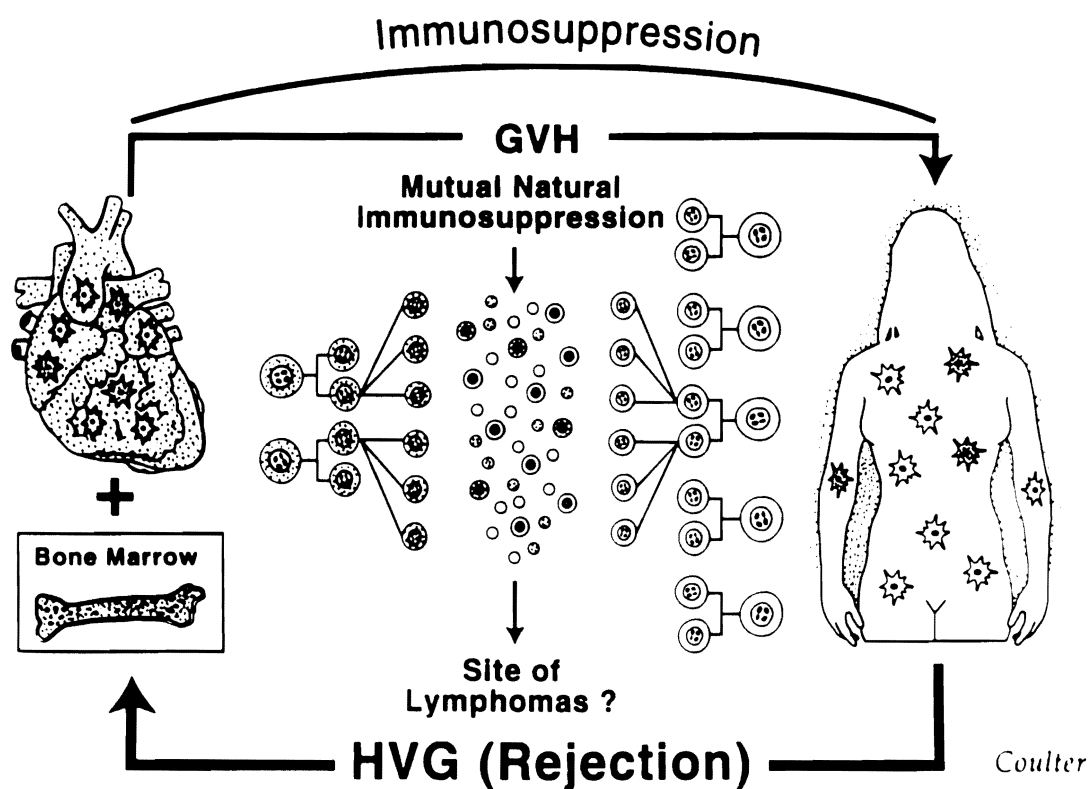
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FIGURE LEGEND

Figure 1 --- Two-way paradigm (organ). Bidirectional mechanism of whole organ graft acceptance involving a graft-vs-host (GVH) reaction by the bone marrow-derived donor leukocytes in the graft that are pitted against the whole recipient immunologic apparatus (host-vs-graft [HVG], rejection). For standard whole organ clinical transplantation, the recipient is not preconditioned. These principles of allograft acceptance apply to xenotransplantation.

Two-Way Paradigm (Heart)



F I G U R E 1